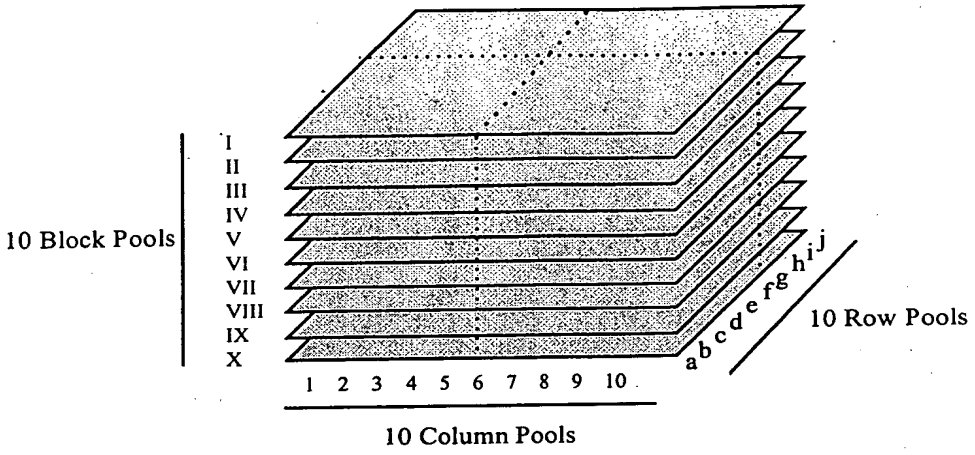
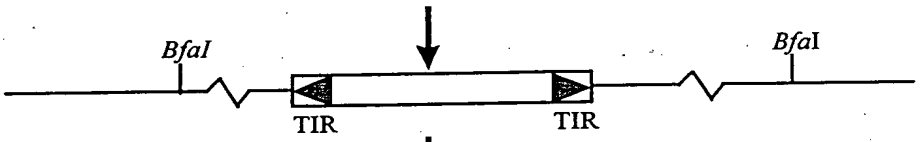
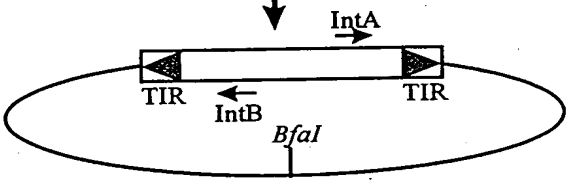
A: Amplification of pools of *dTph1* Flanking sequences



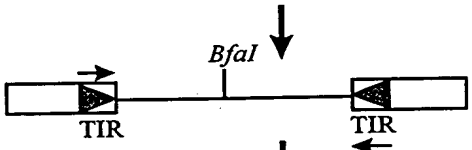
Harvesting of the 30 DNA pools of the 3D library



BfaI digest of the genomic DNA
Backligation of the fragments



iPCR with IntA and IntB primer to generate 30 pools of flanking sequences



Reamplification with nested IR primer

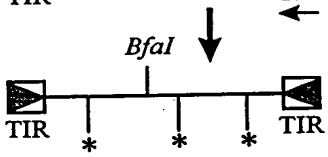


Figure 1A

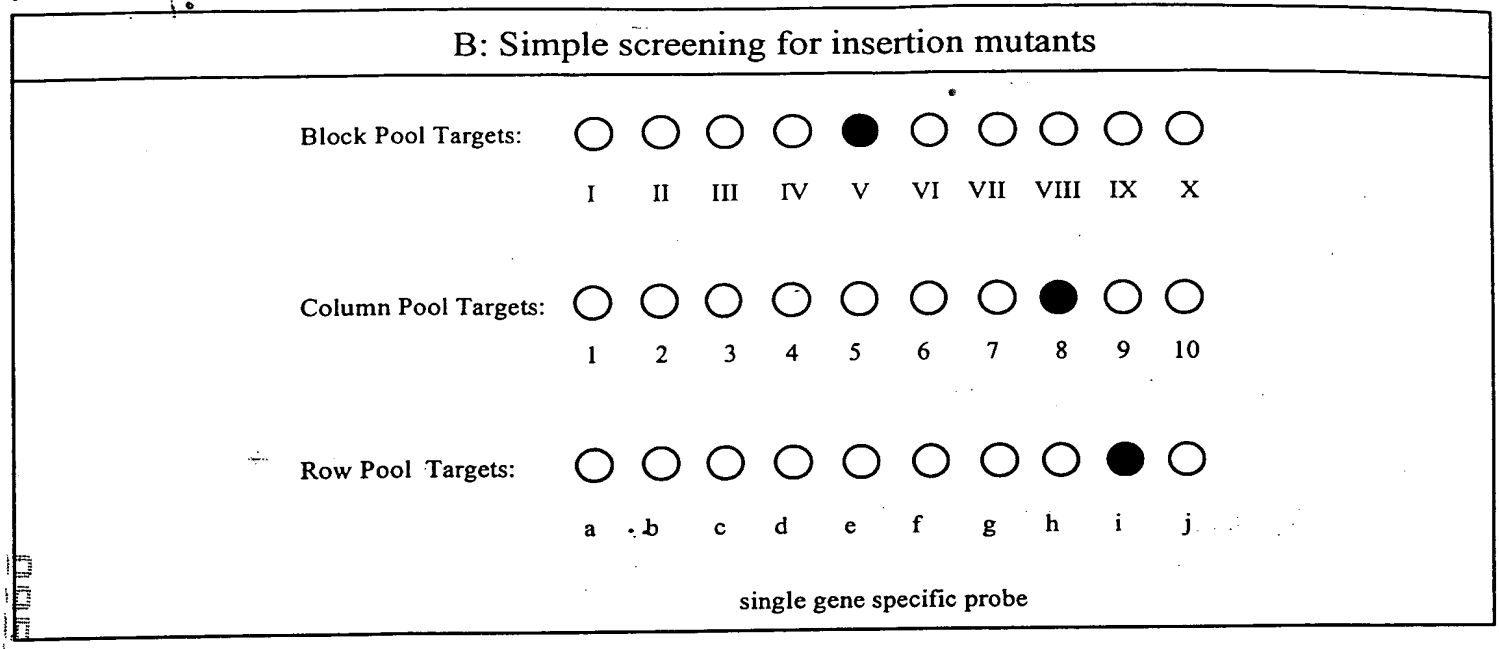


Figure 1B

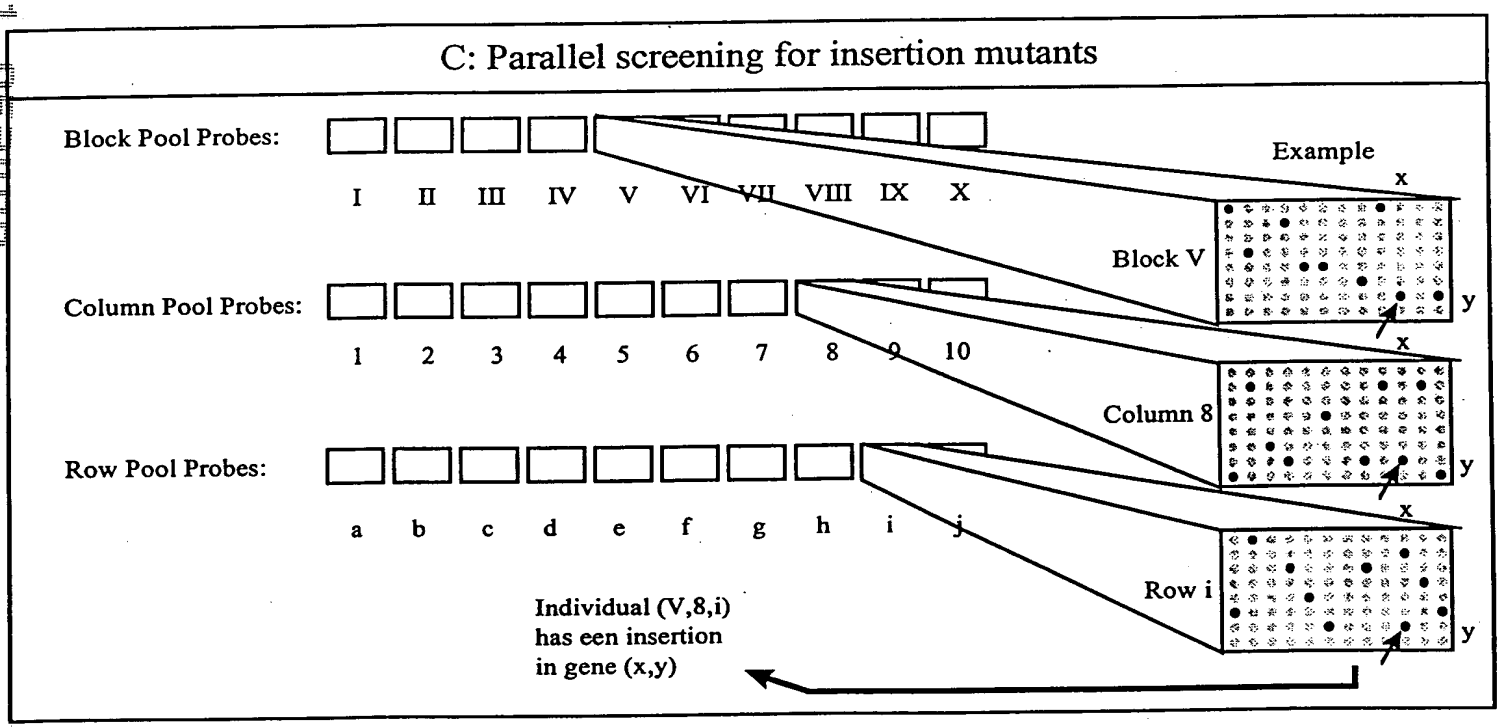
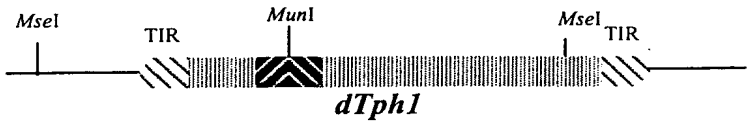
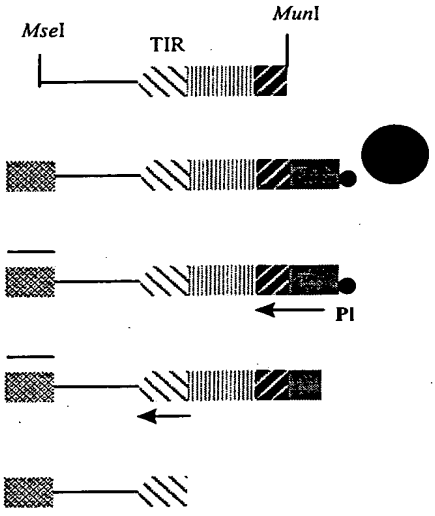


Figure 1c

Figure 1: Parallel screening for insertion mutants using iPCR: Schematical outline of the technique
A: Amplification of the *dTph1* flanking sequences: 1000 plants are organized in a 3D array, and 30 genomic DNA pools are prepared, each representing 100 individual plants. The 30 pools of genomic DNA are digested with a tetracutter enzyme (*Bfa*I). The genomic fragments are self-ligated and the insertion flanking sequences are amplified by PCR using two internal insertion specific primers, IntA and IntB. The specificity of the amplified products can be increased by nested reamplification of the flanking sequences using the terminal inverted repeat primer (TIR).
B: Simple screening for insertion mutants: The amplified flanking sequence pools are displayed on a filter and hybridized with a probe for any target gene of interest in a serial manner.
C: Parallel screening for insertion mutants: The 30 amplified flanking sequence pools are labelled and used to probe an array of target genes displayed on 30 replica filters (or microarrays) in a set of 30 hybridization reactions

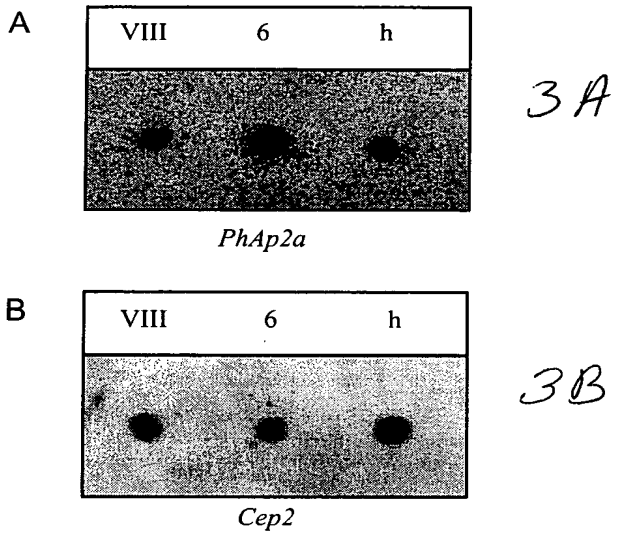
AMPLIFICATION and DETECTION of TRANSPOSON FLANKING SEQUENCES USING TRANSPOSON DISPLAY (After Van den Broeck *et al.*, 1998)





1. Digestion of genomic DNA with MunI and Mse
2. Ligation of biotinylated MunI adaptor and MseI adaptor
3. Pre-amplification using primer based on the MunI adaptor and the insertion element and primer based on the MseI adaptor
4. Re-amplification using primer based on the TIR and the MseI adaptor
5. Insertion element flanking sequences

FIGURE 2

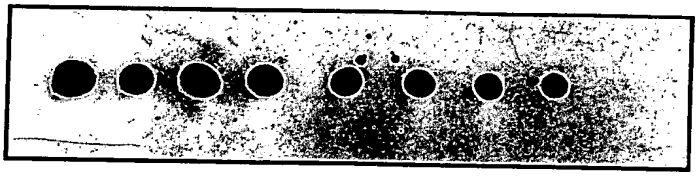


3A

3B

Figure 3: **Generation of a 3D library of flanking sequences**

DNA of an *PhAp2A* insertion mutant was added as a positive control to all the 3D pools, *dTph1* flanking sequences were amplified as desribed. Pools VII, 6, h were spot blotted and hybridized with A: a *PhAp2A* probe; B: a *Cep 2* probe.



Dilution: Mut A B C D E F G Wt

Figure 4a: 3D Screening with a 4Kb XbaI *Ap2A* fragment as a probe. Transposon display fragments spotted; A-G represent a dilution series as described in the text; Wt is wildtype. The probe was fluorescein-labeled, exposure time 5 minutes.

Block III

Row 4

Block I

Figure 4b: **Parallel screening for *Stig 1* mutant**
49 plants (left side of the picture) or 100 plants (right side) were pooled and sampled. Two samples (Block III and Row 4) were known to contain an insertion mutant for the *Stig 1* gene, whereas one sample (Block I) was known not to contain this insert. Six replicate filters were prepared, harboring spotblotted DNA from the *Ap2A* gene (left side of each filter) or the *Stig 1* gene (right side).
The samples mentioned did not contain an insertion for the *Ap2A* gene.
PCR products from each sample, obtained by Transposon Display, were fluorescein-labeled and used as a probe. Exposure time 5 minutes.